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VWF/ADAMTS13 Imbalance, But Not Global Coagulation or Fibrinolysis, Is Associated With Outcome and Bleeding in Acute Liver Failure

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BACKGROUND AND AIMS: Recent studies of acute liver failure (ALF) in man and animals have suggested that rebalanced hemostasis occurs, with distinct hypercoagulable features clinically evidenced by a low risk of bleeding. Rodent models have shown a link between intrahepatic microthrombus formation and progression of ALF. We sought to confirm these earlier findings in a large series of patients with well-characterized ALF from the Acute Liver Failure Study Group.

APPROACH AND RESULTS: Citrated plasma samples taken on admission from 676 patients with ALF or acute liver injury (international normalized ratio ≥2.0 without hepatic encephalopathy) were used to determine levels of von Willebrand factor (VWF), a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) activity, thrombomodulin-modified thrombin generation, and clot lysis time (CLT) and compared with the levels in 40 healthy controls. Patients had 3-fold increased VWF levels, 4-fold decreased ADAMTS13 activity, similar thrombin generating capacity, and 2.4-fold increased CLT, compared with controls. Increasing disease severity was associated with progressively more elevated VWF levels as well as hypofibrinolysis. Patients who died or underwent liver transplantation within 21 days of admission had higher VWF levels, lower ADAMTS13 activity, but similar thrombin generation and a similar proportion of patients with severe hypofibrinolysis, when compared with transplant-free survivors. Likewise, patients with bleeding complications had higher VWF levels and lower ADAMTS13 activity compared to those without bleeding. Thrombin generation and CLT did not differ significantly between bleeding and nonbleeding patients.

CONCLUSIONS: Rebalanced hemostatic status was confirmed in a large cohort of patients with acute liver injury/ALF, demonstrating that VWF/ADAMTS13 imbalance is associated with poor outcome and bleeding. The association between VWF/ADAMTS13 imbalance and bleeding suggests that bleeding in ALF relates more to systemic inflammation than a primary coagulopathy. (HEPATOLOGY 2021;73:1882-1891).

Liver diseases are frequently accompanied by alterations in the hemostatic system, and lead to derangements in routine diagnostic tests of hemostasis. However, although prolongation of the prothrombin time and derived international normalized ratio (INR) and a low platelet count are indicative of a bleeding tendency, clinical and laboratory studies have demonstrated that patients with liver disease are in a hemostatic rebalance due to a simultaneous

Abbreviations: ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; ALF, acute liver failure; ALI, acute liver injury; CI, confidence interval; CLT, clot lysis time; ETP, endogenous thrombin potential; INR, international normalized ratio; pnp, pooled normal plasma; RBC, red blood cell; SIRS, systemic inflammatory response syndrome; TTP, thrombotic thrombocytopenic purpura; VWF, von Willebrand factor.

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decline in prohemostatic and antihemostatic factors.[1] The concept of rebalanced hemostasis was initially developed for patients with cirrhosis, but recent evidence suggests maintenance of hemostatic balance even in the sickest patients, including those with acute-on-chronic liver failure[2] and acute liver failure (ALF).[3] It is, however, plausible that hemostatic balance becomes more susceptible to perturbation with increasing severity of disease or with additional disease complications. For example, the development of acute kidney injury in patients with cirrhosis was recently shown to alter specific hemostatic pathways, which may explain the increased bleeding risk of cirrhosis complicated by acute kidney injury.[4]

Patients with ALF have long been considered to have a hemostasis-related bleeding tendency. An elevated INR is a defining feature of ALF,[5] and ALF is frequently accompanied by abnormalities in other routine laboratory tests of hemostasis, such as a reduced platelet count.[6,7] Although bleeding was reported to be common in these patients in the 1970s,[8-10] recent data from a large cohort of patients with ALF showed that spontaneous, clinically significant bleeding complications are rare,[11] and bleeding complications were extremely uncommon contributors to death. In fact, thromboses may be nearly as common as bleeding complications in patients with ALF.[12] We have previously demonstrated that patients with ALF are characterized by a rebalanced hemostatic status with hypercoagulable elements. Specifically, we demonstrated preserved thrombin-generating capacity, profound hypofibrinolysis, and a von Willebrand factor (VWF)/a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) imbalance.[13,14]

VWF is a multimeric protein that interacts with platelet glycoprotein Ib, which is essential in the formation of a platelet plug. High molecular weight multimers of VWF are the most effective in supporting platelet adhesion. The multimeric size of VWF is regulated by ADAMTS13, which cleaves the large multimers into smaller, less active multimers.[15] The importance of ADAMTS13 activity is evidenced by the severe condition of patients with thrombotic thrombocytopenic purpura (TTP), which is characterized by a deficiency in, or the presence of neutralizing antibodies to, ADAMTS13. The thrombotic manifestations in patients with TTP are related to circulating ultralarge VWF multimers, which spontaneously bind and aggregate platelets.[16] We have previously demonstrated very high plasma levels of VWF with very low ADAMTS13 activity in a group of 50 patients with ALF. High VWF plasma levels are likely a result of chronic endothelial activation, which in turn is likely related to systemic inflammation. We demonstrated that these high VWF levels compensate in part for the thrombocytopenia of ALF.[14] Interestingly, thrombocytopenia in ALF is also related to systemic inflammation,[17] indicating an intricate interplay between inflammation and hemostatic alterations in ALF. In our cohort of 50 patients, we showed a relationship between the imbalance between VWF and ADAMTS13 and outcome of disease.[14]

Observations in animal models have provided a mechanistic explanation for the relationship between a hypercoagulable phenotype and outcome of disease.

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For example, mouse models of ALF have demonstrated profound intrahepatic platelet and fibrin deposition shortly after liver injury.[18,19] Intrahepatic activation of hemostasis may be initiated by severe liver injury, resulting in exposure of thrombogenic surfaces including necrotic cells. In addition, intrahepatic decryption of tissue factor, the natural activator of coagulation, contributes to activation of coagulation.[20] Activation of coagulation appears to drive disease progression, as anticoagulant drugs decrease both intrahepatic fibrin deposition and liver injury.[18,21] In addition, animal models of ALF have shown a direct link between elevated plasma levels of the platelet adhesive protein VWF, intrahepatic platelet accumulation, and progression of the disease.[19] In these experiments, interventions that block VWF-mediated intrahepatic platelet deposition improve repair of the injured liver.

Based on these observations, we hypothesized that, in patients with acute liver injury (ALI) or ALF, a hypercoagulable phenotype may predict poor outcome (death or liver transplantation), whereas a hypocoagulable phenotype may predict bleeding complications. The aim of this study, therefore, was to investigate the baseline hemostatic state in a large cohort of patients with ALI/ALF in relation to bleeding and clinical outcome.

Patients and Methods

Adult patients with ALI or ALF who were enrolled in the Acute Liver Failure Study Group registry between 2011 and 2018 were included from 16 sites across North America. The registry collects detailed demographic, clinical, and biochemical information on admission and daily for 7 days, as well as monitoring all patients to 21-day outcomes. As previously described, ALI was defined as liver injury in a patient with no known previous liver disease, an admission INR of ≥2.0, and a duration of illness of ≤26 weeks in the absence of hepatic encephalopathy.[7] ALF was defined as an acute hepatic illness with INR ≥ 1.5 and the presence of any degree of hepatic encephalopathy. Informed consent was obtained from either the patient or their next of kin, depending on the patient's level of hepatic encephalopathy. Bleeding complications were reported daily by the study site clinical investigators from admission (day 1) through day 7, and were defined as outlined previously.[11] In short, sites were required to specify on each day whether bleeding had occurred, and if so, the site of origin, but no formal definition of bleeding was used. The case report forms for all bleeders was reviewed by the study principal investigator (R.T.S.) and classified as either spontaneous (non-procedure-related) or post-procedural, and further classified according to the site of bleeding. The adjudication process was deliberately designed to be overly inclusive without regard to the severity of bleeding or whether the bleeding episode resulted in red blood cell (RBC) transfusion. Ethical approval for the study was obtained from the study-wide medical ethical committee at the University of Texas Southwestern Medical Center and by the institutional review boards of each participating study site.

Whole blood from patients with ALI/ALF was drawn into 4.5-mL citrated vacutainers on admission to the study at study sites. Platelet-poor plasma was prepared by centrifugation at 1,500*g* for 15 minutes within 2 hours of collection, separated into 0.5-mL aliquots, and frozen at −80°C. Citrated plasma samples from 40 healthy individuals were used to determine reference values for the hemostatic tests in this study. Human pooled normal plasma (pnp) was used for calibration in the performed tests, and was a generous gift from Dr. J. C. Meijers (Amsterdam UMC, the Netherlands).

VWF antigen levels were determined by an enzyme-linked immunosorbent assay using commercially available anti-VWF antibodies (A0082; DAKO, Glostrup, Denmark), as described previously.[22] Human pnp was used for the standard curve. VWF antigen levels in human pnp was set at 100%, and values obtained from patients with ALF were expressed as a percentage of VWF levels in pnp.

ADAMTS13 activity was assessed using the FRETS VWF73 kit (Peptanova, Sandhausen, Germany) according to the manufacturers' instructions with one exception. We pretreated samples with bilirubin oxidase (2.5 U/mL; Sigma-Aldrich, Zwijndrecht, the Netherlands) for 30 minutes at 37°C as described previously.[23] ADAMTS13 activity was calculated as a percentage of the activity measured in human pnp.

A thrombomodulin-modified thrombin generation assay, which assesses the balance between all procoagulant and anticoagulant factors, was performed using calibrated automated thrombinography as described previously.[24] We report the endogenous thrombin

potential (ETP), which represents the area under the thrombin generation curve.

Clot lysis time (CLT) was determined by measuring turbidity changes during clot formation and subsequent lysis of the clot, as described previously.[25]

Descriptive statistics were used to summarize demographics, clinical characteristics, and outcomes of the study population. Continuous variables were reported as means and SDs. Hypercoagulable phenotypes were compared among predefined groups (outcome, etiology, and bleeding status) using Student *t* test. Categorical variables were compared with a chi-squared test or Fisher's exact test when appropriate. All analyses were done using SAS 9.4 (SAS Institute, Inc., Cary, NC) with a two-sided significance level of 0.05. Statistical *P* values were not adjusted for multiple testing.

Results

PATIENT CHARACTERISTICS

Citrated plasma collected on admission was available from a total of 676 patients with ALI/ALF recruited from the ALF Study Group registry. Of these, 308 (45.6%) had acetaminophen-induced ALI/ALF, 50 (7.4%) had bleeding complications, and 483 survived without liver

TABLE 1. Demographic, Laboratory, and Clinical Data of the Cohort of Patients With ALI/ALF

Variable	Patients With ALI (n = 297) n (%) or mean ± SD	Patients With ALF (n = 379) n (%) or mean ± SD	Patients With ALI/ALF (n = 676) n (%) or mean ± SD
Demographics			
Age (years)	38.6 ± 15.4	42.4 ± 15.9	40.8 ± 15.8
Gender (female)	176 (59.3)	242 (63.9)	418 (61.8)
Race (Caucasian)	225 (75.8)	278 (73.5)	503 (74.4)
APAP etiology	164 (55.2)	144 (38.0)	308 (45.6)
Laboratory Data on Hospital Admission			
Platelet count ($\times 10^9$/L)	155.9 ± 80.9	137.0 ± 86.7	145.2 ± 86.7
INR	2.8 ± 1.3	3.6 ± 1.9	3.3 ± 1.7
WBC ($\times 10^9$/L)	8.4 ± 4.3	12.2 ± 8.1	10.5 ± 6.9
Albumin (g/dL)	3.0 ± 0.6	2.7 ± 0.6	2.8 ± 0.6
Lactate (mg/dL)	2.6 ± 1.9	4.7 ± 3.9	4.0 ± 3.5
Creatinine (mg/dL)	1.3 ± 1.2	2.1 ± 1.7	1.7 ± 1.6
Phosphate (mg/dL)	2.7 ± 1.4	3.5 ± 1.9	3.2 ± 1.8
Bicarbonate (mg/dL)	23.6 ± 4.0	21.1 ± 5.4	22.1 ± 5.0
Clinical Features on Hospital Admission			
Hepatic encephalopathy (high grade) (%)	0 (0.0)	170 (44.9)	170 (25.2)
SIRS (%)	93 (31.3)	240 (63.3)	333 (49.3)
Infection (%)	21 (7.1)	63 (16.6)	84 (12.4)
Anticoagulants (%)	11 (3.7)	21 (5.5)	32 (4.7)
Interventions After Admission			
RBC transfusion (%)	10 (3.4)	82 (21.6)	92 (13.6)
RRT (%)	18 (6.1)	153 (40.4)	171 (25.3)
Vasopressors (%)	14 (4.7)	158 (41.7)	172 (25.4)
Outcome			
Bleeding (%)	10 (3.4)	40 (10.6)	50 (7.4)
21-day transplant-free survival (%)	273 (91.9)	210 (55.4)	483 (71.5)
Liver transplantation within 21 days (%)	9 (3.0)	62 (16.4)	71 (10.5)
Death within 21 days (%)	15 (5.1)	112 (29.6)	127 (18.8)

Abbreviations: APAP, acetaminophen; RRT, renal replacement therapy; and WBC, white blood cell.

transplantation at 21 days after registry enrollment (transplant-free survivors, 71.5%). Demographic, laboratory, and clinical data of all patients are listed in Table 1.

PATIENTS WITH ALI/ALF HAVE UNBALANCED VWF/ADAMTS13, NORMAL COAGULATION POTENTIAL, AND ARE IN A HYPOFIBRINOLYTIC STATE

Hemostatic features of patients with ALI/ALF on admission to the study were compared with those in healthy controls. VWF levels were 3 times higher in patients than in healthy controls (448.0% ± 220.8 vs. 155.0% ± 58.9 [mean ± SD]). ADAMTS13 activity was 4 times lower in patients than in healthy controls (21.1% ± 23.2 vs. 80.9% ± 33.1). Thrombin generating capacity, assessed by thrombomodulin-modified calibrated automated thrombinography, was similar in patients compared with healthy controls (550.2 nM*min ± 388.7 vs. 565 nM*min ± 352). Plasma CLT was 2.4-fold higher in patients than in healthy controls (143 minutes ± 45 vs. 60 minutes ± 9). However, many of the samples of patients with ALI/ALF did not lyse within the 180 minutes the assay is run, and were arbitrarily assigned a CLT of 180 minutes for these analyses; of the 641 patients of whom CLT values were available, 413 (64.4%) had CLT > 180 minutes, whereas in healthy controls none had a CLT > 180 minutes.

VWF/ADAMTS13 IMBALANCE AND FIBRINOLYTIC STATUS ARE ASSOCIATED WITH SEVERITY OF ILLNESS

We next assessed the relationship between hemostatic features of patients with ALI/ALF on admission and clinical and laboratory markers of severity of disease. Patients with acetaminophen-induced ALI/ALF had lower VWF levels and higher ADAMTS13 activity than patients with other etiologies of the disease (Table 2). No significant difference was observed in ETP between acetaminophen-induced and non-acetaminophen-induced ALI/ALF, but CLT > 180 minutes was more frequent in patients with acetaminophen-induced ALI/ALF than patients with other etiologies (Table 2). Patients with more severe ALI/ALF, as evidenced by higher grade of hepatic encephalopathy, requirement for vasopressors or renal replacement therapy, presence of systemic inflammatory response syndrome (SIRS), INR prolongation, and abnormal plasma levels of lactate, creatinine, phosphate, and bicarbonate had higher levels of VWF (Table 2). Severity of disease was not associated with increased ETP, with the exception of increasing grade of hepatic encephalopathy and abnormal plasma levels of creatinine and phosphate (Table 2). CLT > 180 minutes was more frequent in patients with higher severity of disease (Table 2).

VWF/ADAMTS13 IMBALANCE IS ASSOCIATED WITH OUTCOME IN PATIENTS WITH ALI/ALF

Patients who did not undergo liver transplantation or die within 21 days of study admission were defined as transplant-free survivors. As indicated in Table 3, non-transplant-free survivors had higher VWF levels and lower ADAMTS13 activity than transplant-free survivors. Thrombin generation did not differ significantly, and the proportion of patients with a CLT > 180 minutes was similar between these groups (Table 3).

BLEEDING COMPLICATIONS ARE ASSOCIATED WITH ELEVATED VWF LEVELS AND DECREASED ADAMTS13 ACTIVITY

Bleeding complications were defined as spontaneous or procedure-related bleeding in the first 7 days of admission to the study,[11] and occurred in 50 of 676 patients (7.4%). As indicated in Table 4, VWF levels were higher and ADAMTS13 activity was lower in patients with bleeding complications than in patients without bleeding complications. No significant differences were observed in ETP and CLT between bleeding and nonbleeding patients (Table 3). The proportion of patients with a CLT > 180 minutes was similar between patients with and without bleeding complications (Table 4). Patients who received RBC transfusions had higher VWF levels than patients who did not receive RBC transfusions (Table 5). However, ADAMTS13 activity and ETP were not significantly different between these groups. In addition, the proportion of patients with a CLT > 180 minutes was similar between patients with and without RBC transfusion (Table 5).

TABLE 2. Hemostatic Features of Patients With ALI/ALF on Study Admission in Relation to Clinical and Laboratory Data

Complications of ALI/ALF		VWF (%) (95% CI)	ADAMTS13 (%) (95% CI)	ETP (nM IIa*min) (95% CI)	CLT > 180 Minutes (%) (95% CI)
APAP etiology	Yes (n = 308)	400.6 (380.6-420.5)	23.8 (20.9-26.7)	529.7 (491.8-567.7)	76.7 (71.8-81.5)
	No (n = 368)	487.9 (462.7-513.0)‡	18.9 (16.7-21.0)*	567.8 (522.9-612.6)	53.9 (48.6-59.2)†
Hepatic encephalopathy	Grade 0/1/2 (n = 506)	418.1 (400.2-436.1)	22.4 (20.3-24.6)	529.1 (498.6-559.5)	59.3 (54.8-63.7)
	Grade 3/4 (n = 170)	536.9 (500.0-573.7)‡	17.3 (14.4-20.2)*	613.7 (537.3-690.2)‡	80.0 (73.7-86.3)†
Use of vasopressors	Yes (n = 172)	534.0 (498.7-569.3)	17.5 (14.7-20.2)	568.6 (489.4-647.8)	75.6 (69.0-82.3)
	No (n = 504)	418.4 (400.1-436.7)‡	22.4 (20.2-24.6)*	543.9 (514.4-573.3)	60.6 (56.2-65.0)†
RRT	Yes (n = 171)	494.7 (464.7-524.7)	16.8 (13.8-19.7)	541.0 (460.8-621.3)	81.5 (75.4-87.5)
	No (n = 505)	432.1 (412.2-451.9)‡	22.6 (20.5-24.8)*	553.4 (524.3-582.6)	58.7 (54.2-63.1)†
Infection	Yes (n = 168)	468.4 (434.6-502.2)	21.1 (17.6-24.6)	568.9 (513.1-624.6)	65.2 (57.8-72.7)
	No (n = 508)	441.2 (422.0-460.5)	21.1 (19.1-23.2)	544.0 (508.8-579.2)	64.2 (59.9-68.5)
SIRS	Yes (n = 333)	499.2 (472.9-525.6)	19.0 (16.8-21.1)	547.0 (501.6-592.4)	75.6 (70.9-80.4)
	No (n = 313)	391.4 (371.5-411.2)†	24.2 (21.1-27.1)*	556.5 (516.1-596.8)	53.4 (47.4-59.1)†
Platelet count ($\times 10^9$/L)	0-100 (n = 208)	464.5 (433.2-495.9)	17.6 (15.0-20.2)	556.1 (508.3-604.0)	72.5 (66.1-78.8)
	>100 (n = 444)	442.1 (421.8-462.4)	23.1 (20.8-25.4)*	550.1 (510.9-589.3)	61.0 (56.3-65.7)*
INR	<1.5 (n = 23)	323.7 (259.3-388.0)	22.1 (15.5-28.7)	478.3 (309.1-647.4)	34.8 (13.7-55.8)
	≥1.5 (n = 626)	450.3 (432.8-467.8)‡	21.4 (19.6-23.3)	547.9 (519.5-576.3)	64.9 (61.0-68.7)*
Lactate	Normal (n = 115)	374.6 (340.0-409.2)	24.9 (20.3-29.4)	567.1 (504.4-629.9)	63.6 (54.5-72.8)
	Abnormal (n = 245)	492.1 (465.9-518.3)‡	21.0 (18.2-23.9)	588.0 (528.2-647.9)	80.1 (74.9-85.3)*
Creatinine	Normal (n = 358)	393.8 (373.9-413.7)	24.5 (21.9-27.1)	586.5 (543.5-629.5)	52.9 (47.6-58.2)
	Abnormal (n = 308)	506.2 (480.1-532.4)‡	17.5 (15.2-19.8)†	514.9 (473.5-556.3)‡	77.4 (72.6-82.3)†
Phosphate	Normal (n = 447)	420.9 (403.0-438.8)	23.5 (21.1-25.8)	574.6 (536.4-612.8)	62.5 (57.9-67.1)
	Abnormal (n = 80)	528.3 (478.2-578.4)‡	13.6 (9.9-17.3)†	461.0 (378.4-543.7)‡	84.0 (75.5-92.5)†
Bicarbonate	Normal (n = 318)	420.1 (396.5-443.6)	20.8 (18.0-23.6)	574.7 (531.2-618.3)	58.0 (52.4-63.6)
	Abnormal (n = 241)	487.7 (458.2-517.3)‡	23.0 (20.1-25.9)	517.5 (475.9-559.1)	76.0 (70.4-81.6)†

Note: Data are presented as mean (95% CI) or percentage (95% CI).
*$P < 0.01$.
†$P < 0.001$.
‡$P < 0.05$.
Abbreviations: APAP, acetaminophen; CI, confidence interval; RRT, renal replacement therapy.

TABLE 3. Hemostatic Features of Patients With ALI/ALF According to 21-Day Outcome: Transplant-Free Survivors and Non-Transplant-Free Survivors (Death or Liver Transplantation)

	Number Analyzed	21-Day TFS (n = 483)	Number analyzed	Non-21-Day TFS (n = 193)
VWF (%) (95% CI)*	480	403.6 (387.1-420.0)	192	559.0 (521.5-596.6)
ADAMTS13 (%) (95% CI)†	478	22.5 (20.3-24.6)	192	17.8 (15.0-20.7)
ETP (nM IIa*min) (95% CI)	471	543.7 (511.8-575.6)	185	566.9 (498.9-635.0)
CLT > 180 minutes (%) (95% CI)	460	65.7 (61.3-70.0)	181	61.3 (54.2-68.5)

Note: Data are presented as mean (95% CI) or percentage (95% CI).
*$P < 0.001$.
†$P < 0.05$.
Abbreviation: TFS, transplant-free survivor.

Discussion

In this study, we confirm and extend our previous findings in 50 patients, that hemostasis in ALI/ALF is characterized by VWF/ADAMTS13 imbalance, normal thrombin generating potential, and hypofibrinolysis.[13,14] In addition, more severe VWF/ADAMTS13 imbalance, but not coagulation or fibrinolytic capacity, was associated with worsening disease outcome and more bleeding complications.

Based on extensive data from animal models of ALF,[18,19] we hypothesized that the hemostatic state

TABLE 4. Baseline Hemostatic Features of Patients With ALI/ALF According to the Occurrence of Bleeding Complications During the First 7 Days of Study Admission

	Number Analyzed	No Bleeding Complications (n = 626)	Number Analyzed	Bleeding Complications (n = 50)
VWF (%) (95% CI)*	622	441.9 (424.5-459.4)	50	523.2 (467.1-579.3)
ADAMTS13 (%) (95% CI)†	621	21.6 (19.7-23.5)	49	15.1 (10.9-19.2)
ETP (nM IIa*min) (95% CI)	607	543.4 (517.3-569.6)	49	634.3 (394.3-874.4)
CLT > 180 minutes (%) (95% CI)	597	64.3 (60.5-68.2)	44	65.9 (51.3-80.5)

Note: Data are presented as mean (95% CI) or percentage (95% CI).
*$P < 0.05$.
†P < 0.01.

TABLE 5. Baseline Hemostatic Features of Patients With ALI/ALF According to the Receipt of RBC Transfusions During the First 7 Days of Study Admission

	Number Analyzed	No RBC Transfusion (n = 584)	Number Analyzed	RBC Transfusion (n = 92)
VWF (%) (95% CI)*	580	438.6 (420.6-456.7)	92	506.9 (463.4-550.3)
ADAMTS13 (%) (95% CI)	578	21.2 (19.3-23.1)	92	20.7 (16.2-25.1)
ETP (nM IIa*min) (95% CI)	567	555.2 (525.0-585.3)	89	518.8 (410.8-626.8)
CLT > 180 minutes (%) (95% CI)	552	64.1 (60.1-68.1)	89	66.3 (56.3-76.3)

Note: Data are presented as mean (95% CI) or percentage (95% CI).
*$P < 0.01$.

of patients with ALF would be related to outcome of disease, with a hypercoagulable phenotype related to poor outcome (death or liver transplantation in the first 21 days of admission). Our results show that a hypercoagulable VWF/ADAMTS13 imbalance, but not thrombin-generating capacity or fibrinolytic status, is associated with poor outcome. Our findings are comparable to our first study in 50 patients with ALI/ALF, in which we showed decreased ADAMTS13 activity to be associated with disease severity and progression, which we attributed to intrahepatic platelet-induced microthrombus formation.[14] Interestingly, although ADAMTS13 levels are very low and even undetectable in a proportion of patients, our previous work has suggested that it is unlikely that thrombotic events in ALF proceed via a mechanism similar to that observed in patients with TTP who have an isolated ADAMTS13 deficiency. Specifically, despite a profound VWF/ADAMTS13 imbalance, we did not find evidence for ultralarge VWF multimers that are causing the thrombotic events in patients with TTP, which we attributed to VWF proteolysis by other circulating enzymes, or by *N*-acetylcysteine, which is known to reduce VWF multimeric size.[14] Nevertheless, as VWF/ADAMTS13 imbalance, but not coagulation or fibrinolytic status, is related to outcome, our studies do suggest that intrahepatic thrombosis and ultimately progression of ALF in humans is dominated by platelet accumulation. We speculate that ADAMTS13 deficiency can promote local, intrahepatic, thrombus growth in a mechanism independent of ultralarge VWF.[26] Conversely, animal studies suggested that thrombin activation and fibrinolysis also contribute to disease progression.[18] It may be that, in humans with ALF, platelets are the dominant constituent of intrahepatic microthrombi, with relatively little fibrin deposition. Alternatively, it may be that the extent of intrahepatic fibrin deposition is independent of the exact coagulation and fibrinolyic potential. Indeed, the normal coagulation potential with a hypofibrinolytic state may facilitate "optimal" intrahepatic fibrin deposition in all patients. It may also be, however, that our laboratory tests are insufficiently sensitive to capture the coagulation and fibrinolytic capacity, which is modulated in plasma by constituents outside plasma, such as blood cells and the vascular endothelium. Nevertheless, the tests used have shown clinical relevance in the context of macrovascular thrombosis.[27,28] Future research in the composition of microthrombi in patients with ALI/

ALF should be performed to gain more insight into the mechanisms of microthrombus formation within the acutely injured liver.

A second hypothesis we explored in this study was that a hypocoagulable state in patients with ALF may be related to bleeding complications. We showed that patients with bleeding complications had higher VWF levels and lower ADAMTS13 activity than patients without bleeding complications, features that suggest a thrombotic rather than a bleeding tendency. In addition, we showed that coagulation and fibrinolytic potential was not related to bleeding. Previous research with the US ALF Study Group showed that bleeding complications in patients with ALF generally occurred in patients who were more systemically ill than nonbleeders and had more extrahepatic dysfunction.[11] Importantly, most bleeding complications were upper gastrointestinal bleeds that are likely unrelated to hemostatic failure, but rather a consequence of "stress-related mucosal disease," a manifestation of critical illness characterized by intense systemic inflammation.[29] We also demonstrated that thrombocytopenia is associated with the development of SIRS and multi-organ system failure.[17]

In systemic inflammation, endothelial cells are activated and release VWF, a response that has been described in patients with chronic liver failure.[30] Furthermore, high VWF levels are associated with portal hypertension and predict severity and outcome in chronic liver disease.[31-33] We propose that the relationship between VWF/ADAMTS13 and clinical bleeding is unrelated to hemostasis, but rather that high VWF levels and low ADAMTS13 activity are merely markers of systemic inflammation in patients with ALI/ALF. In addition, our data suggest that VWF levels, available in most larger diagnostic laboratories, have relevant prognostic value in acute liver disease, and the utility of VWF in the clinical care of these patients should be further explored.

The severity of disease was associated with elevated levels of VWF and hypofibrinolysis, but not with ADAMTS13 activity or ETP (table 2). The absence of relationship between disease severity and ETP has previously been shown in patients with acute liver disease,[13,34] and add to evidence of rebalanced hemostasis independent of disease severity. Although the hypofibrinolytic status was associated with severity of disease (table 2), there was no relation between CLT > 180 minutes and poor outcome. Previous research showed that patients with ALF have significantly increased plasma levels of plasminogen activator inhibitor 1 and decreased levels of thrombin activatable fibrinolysis inhibitor,[13] which are major contributors to a hypofibrinolytic state.[35] We also showed plasminogen activator inhibitor 1 levels to be higher in patients with acetaminophen-induced ALI/ALF,[13] which is in line with a more profound hypofibrinolytic state in these patients. Despite a more hypofibrinolytic state, patients with acetaminophen-induced ALI/ALF have better prognosis than patients with other etiologies of disease.[36] The more profound hypofibrinolytic state in patients with acetaminophen-induced ALI/ALF, but better outcome compared to patients with ALI/ALF of other etiologies, thus likely explains the discrepancy between a link between hypofibrinolysis and disease severity and a lack of predictive value of hypofibrinolysis for outcome.

Although the hemostatic status of patients with ALF appears unrelated to bleeding risk, there may be a link to thrombotic complications, which are not uncommon in this group.[12] Recent data from an ALF Study Group substudy of 200 patients showed an incidence of thrombotic complications of 6% (Stravitz, Durkalski, Lisman, Lee, et al., unpublished data). Although we have previously argued that patients with ALF are characterized by a hemostatic rebalance,[3] there are distinct hypercoagulable features that may dispose to thrombosis. Indeed, both a VWF/ADAMTS13 imbalance and a hypofibrinolytic state have been linked to thrombotic risk in the general population.[27,37] Before the study being referred to, thrombotic complications were not systematically captured in the ALF Study Group registry. Nonetheless, our data confirm that there is a potential hazard in using fresh frozen plasma to correct abnormal laboratory tests of hemostasis, such as an elevated INR, as this may further increase the risk of thrombotic complications.

The data presented represent the extensive experience of a large, multicenter consortium assembled, in part, to explore hemostasis as it relates bleeding complications and clinical outcome of ALI/ALF, and to dispel notions that such patients have a bleeding diathesis. It seems clear, finally, that ALF is characterized by a generally normal hemostatic state but with hypercoagulable elements. These findings enforce previous recommendation for a restrictive

use of prohemostatic agents such as platelet concentrate, fresh frozen plasma, and fibrinogen concentrate, which may further increase hypercoagulable features and worsen intrahepatic activation of hemostasis.[3] Administration of pro-hemostatic agents should therefore preferably be limited to specific situations, such as insertion of intracranial pressure monitors or active bleeding, and should be performed with great caution. In these cases, hemostatic management by coagulation factor concentrates may be preferable to an approach using platelet concentrates and fresh frozen plasma, as the concentrate approach results in a more potent improvement of hemostatic status[38] and may be associated with fewer side effects, as evidenced by experience with this approach in liver transplant surgery.[39]

We acknowledge limitations to our conclusions. First, we performed assays in plasma, omitting blood and endothelial cell contributions. Collection of such whole-blood samples would be challenging in a large multicenter study. Second, we assessed the hemostatic status of patients with ALI/ALF only on admission; therefore, changes in the hemostatic system during the course of the syndrome were not captured. However, previous studies showed that hemostatic potential remains remarkably constant over the course of ALI/ALF,[3,13] and avoid the confounders of intervention by clinicians, such as blood product transfusion.

In conclusion, the hemostatic state in a large cohort of patients with ALI/ALF is characterized by a VWF/ADAMTS13 imbalance, normocoagulability and hypofibrinolysis, features that do not suggest a bleeding tendency. VWF/ADAMTS13 imbalance was associated with poor outcome and with bleeding complications. We propose that intrahepatic platelet thrombus formation as a result of VWF/ADAMTS13 imbalance may drive progression of liver injury, as we previously showed in mouse models of ALF. Interventions aimed at improving the VWF/ADAMTS13 imbalance may be beneficial in the management of ALF and deserve clinical application. Interestingly, *N*-acetylcysteine, which is widely used in the management of ALF, has been shown to reduce VWF multimeric size,[40] and it will be of interest to assess whether this mechanism contributes to the beneficial effects of *N*-acetylcysteine in patients with ALF. Of note, agents targeting VWF and a recombinant ADAMTS13 are currently in clinical development, and although both are antihemostatic agents, they appear to have a very low risk of inducing bleeding.[37,41,42]

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